TABLE III

KINETIC COMPARISON OF NUCLEOPHILES IN THEIR REACTION WITH *p*-NITROPHENYL ACETATE

Catalyst	Reaction conditions	$k_c \times 10^2$, l./mole sec.	log k_c	pK_a of catalyst	Ref.
Imidazole	26.2°, 5% dioxane	46.9	−0.323	7.04	2
Mercaptobenzoate	25°, phosphate buffer	100	0	8.4	[a]
$XC_6H_4O^-$	30°, 28.5% EtOH-H_2O	3	−1.523	8.4	[b]
Salicylate	25°, phosphate buffer	...	.....	13	[a]

[a] This investigation. [b] Calculated from the data of T. C. Bruice and R. Lapinski, THIS JOURNAL, 80, 2622 (1958).

corresponding to equation 2, an ammonium ion facilitates the attack of the carboxylate ion.

The efficiency of the catalyst *o*-mercaptobenzoic acid does not approach that of the enzymes papain and ficin. However, a comparison, shown in Table III, of the nucleophilicities of *o*-mercaptobenzoic acid and other compounds in reaction with *p*-nitrophenyl acetate reveals that *o*-mercaptobenzoic acid is indeed a powerful nucleophile. For example, *o*-mercaptobenzoic acid is a better nucleophile than a phenoxide ion of comparable pK_a. Furthermore *o*-mercaptobenzoic acid is a more effective catalyst around neutrality than salicylate because the latter does not form an appreciable amount of dianion at neutral *p*H's.

A further analogy between the model system and the enzyme action is the formation of an acyl-catalyst compound which upon intramolecular reaction leads to the products of reaction and the regeneration of the catalyst. The two-step process envisaged for the enzyme was first suggested by Smith,[13] but has been criticized by him recently on the grounds that it is thermodynamically unsound.[8] He has instead suggested that the active site in papain is not a sulfhydryl group and a carboxylate group but a combination of these of higher free energy, namely, a thiol ester. It is difficult to assess the thermodynamic argument because of the paucity of data available for the cases under question. It should be pointed out that any mechanism for catalysis of ester hydrolysis by a thiol ester is extremely complicated and probably must involve another nucleophilic group.

CHICAGO 16, ILL.

[CONTRIBUTION FROM THE SCHOOL OF PHARMACY, UNIVERSITY OF WISCONSIN]

Kinetics and Mechanism of Formation of Sulfonate from Epinephrine and Bisulfite

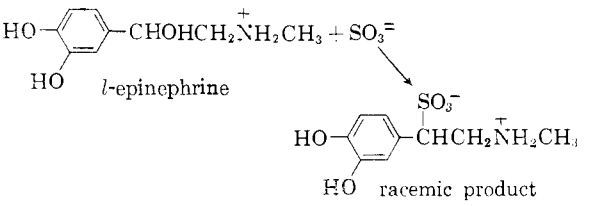
BY TAKERU HIGUCHI AND LOUIS C. SCHROETER

RECEIVED AUGUST 5, 1959

The over-all mechanism of the reaction between epinephrine and bisulfite leading to formation of a sulfonate has been studied. At *p*H values above 5 the reaction appears to be a simple SN2 reaction involving direct attack of sulfite ion on the catecholamine with an apparent heat of activation of 24 kcal. mole^{-1}. Below *p*H 5 there appears to be a parallel SN1 reaction which appears to pass through the same activated intermediate responsible for racemization of epinephrine exhibiting zero-order dependency on bisulfite or sulfite concentration. The rate expression developed on these assumptions has been found to approximate closely the observed *p*H profile.

Although some kinetic data already have been reported on the reaction observed between *l*-epinephrine and sodium bisulfite in aqueous solution, the over-all mechanism of this unexpected reaction has not been discussed in earlier publications. In the present communication it is shown that the rate of the observed reaction can best be explained on the basis of a combination of SN1 and SN2 reactions. The first-order reaction, according to our findings, seems to be directly involved with the racemization mechanism of the catecholamine. The bimolecular reaction appears to be parallel but independent of the other.

Previous studies have shown that *l*-epinephrine is gradually lost from aqueous solutions under nitrogen containing the drug and sodium sulfite between *p*H 4–7. The sulfur compound is normally present in aqueous preparations of the drug as an antioxidant. The isolated end-product and the stoichiometry of the reaction indicate the over-all reaction as illustrated in the formula diagram. The isolated product, m.p. 259° dec., has been found to be both optically and physiologically inactive.[1]



Experimental

Epinephrine solutions were prepared using synthetic *l*-epinephrine hydrochloride in acetate, phthalate or phosphate buffers of varying ionic strength. The *p*H was determined at 25°. Solutions were flushed with nitrogen and stored under a positive nitrogen atmosphere until ready for use. These solutions served as blanks. Identical solutions were prepared containing, in addition, varying concentrations of sodium bisulfite or sodium sulfite. The *p*H of these solutions was adjusted at 25° to that of the appropriate blank and then flushed with nitrogen.

Solutions employed in polarimetric studies were filled into a jacketed polarimeter tube designed so that a nitrogen atmosphere was maintained above the solution at all times. Polarimetric measurements were made on the reacting system with a Zeiss–Winkel polarimeter using 589 mμ filtered sodium light at the temperature of the thermostat. Tem-

(1) L. C. Schroeter, T. Higuchi and E. Schuler, *J. Am. Pharm. Assoc.*, **47**, 723 (1958).

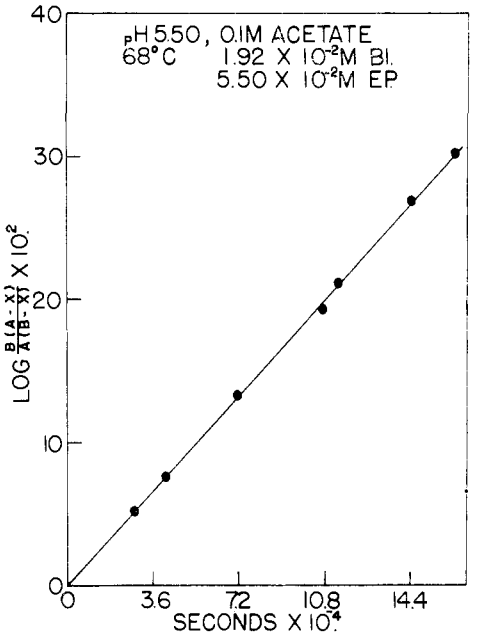


Fig. 1.—Second-order plot of epinephrine–sodium bisulfite reaction at *p*H 5.50: A, 5.50 × 10^{-2} *M* epinephrine (Ep); B, 1.92 × 10^{-2} *M* sodium bisulfite.

perature of the polarimeter tube was maintained within ± 0.1° of the thermostat as described previously.[2]

Solutions used for chemical degradation studies were rapidly filled into hard glass ampules. The filled ampules were flushed with nitrogen, evacuated, and sealed under vacuum. The sealed ampules were stored in a thermostat in which the temperature variation was less than 0.1°. Sampling was performed by periodically removing ampules from the thermostat and quickly chilling in an ice–water mixture. Prior to analysis, ampules were thermostated briefly at 25°. The *p*H of all solutions subjected to elevated temperature was determined at 25°. Available bisulfite was determined by iodometric titration. Chemical loss of epinephrine was determined by a modified triacetyl derivative procedure.[3]

Results and Discussion

These studies indicate that epinephrine in solution above *p*H 5 reacts with sulfite ion following largely a second-order mechanism. This is evident in Fig. 1, in which the reaction was followed iodometrically for bisulfite disappearance. Values were plotted to give a linear relationship for reactions obeying the second-order law. Similar linear second-order plots were observed at *p*H's of 6.50 and 7.00. The equivalence of loss of optical activity attributed to *l*-epinephrine and bisulfite at these *p*H values is evident in the results shown in Fig. 2. For this run equivalent amounts of the reactants were used and the second-order plot based on this relationship yields the same straight line from both sets of results.

The apparent heat of activation for this reaction was determined at *p*H 6.50 in a phosphate buffer. Since the second-order rate constant is an invert linear function of the reciprocal of the absolute temperature, the value of 24 kcal. mole^{-1} was estimated from this plot. Although ionic strength unfortunately was not maintained constant in these studies, it is felt that its effect was only of secondary importance.

(2) L. C. Schroeter and T. Higuchi, *J. Am. Pharm. Assoc.*, **47**, 426 (1958).

(3) T. Higuchi, T. D. Sokoloski and L. C. Schroeter, *ibid.*, **48**, 553 (1959)

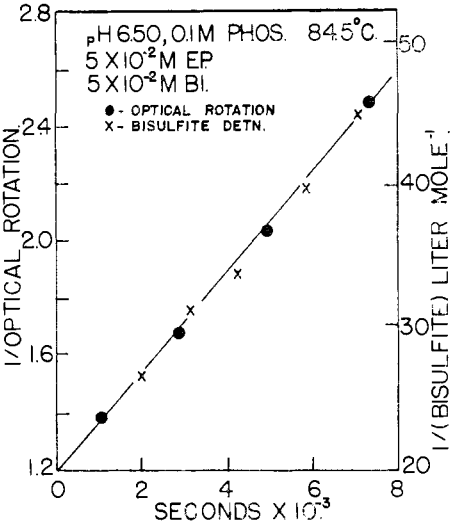


Fig. 2.—Reciprocal bisulfite (Bi) concentration in liter mole^{-1} shown on right scale; reciprocal optical activity indicated on left.

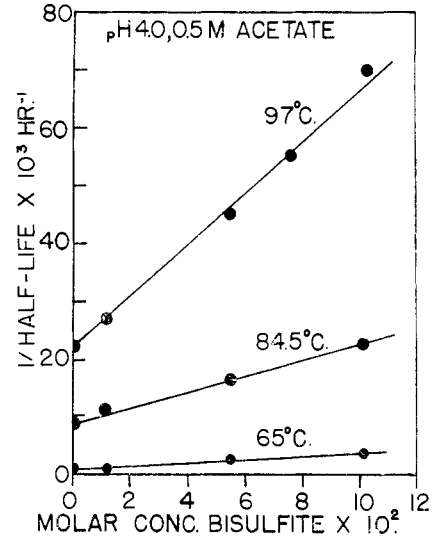


Fig. 3.—Reciprocal half-life of optical activity of epinephrine (Ep) solutions at *p*H 4.0 plotted against bisulfite concentration in moles per liter.

The influence of bisulfite concentration on the rate of loss of the catecholamine from solution under more acidic conditions than those reported above indicates, however, that the over-all kinetics of the reaction cannot be explained adequately simply on the basis of a second-order (SN2) reaction. In studies reported earlier[1] it was shown, for example, that at *p*H 4.5 and 80° the presence of bisulfite largely repressed formation of *d*-epinephrine from *l*-epinephrine by racemization although this reaction occurs at a reasonable rate in absence of the antioxidant. Although the optical activity of the solution of the catecholamine is lost progressively in the presence of bisulfite, the loss has been shown by chemical analysis to be due to formation of the optically inactive sulfonate.

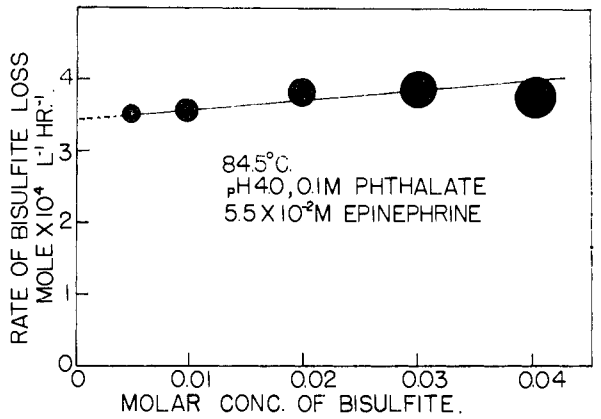


Fig. 4.—Rate of bisulfite loss at *p*H 4.0 and 84.5° in mole liter^{-1} hr.$^{-1}$ as a function of bisulfite concentration.

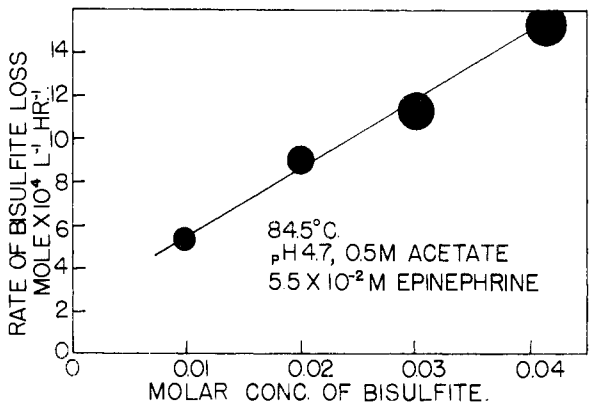


Fig. 5.—Rate of bisulfite loss at *p*H 4.7 and 84.5° in mole liter^{-1} hr.$^{-1}$ as a function of bisulfite concentration.

The general dependency of the rate of loss of over-all optical activity of a *l*-epinephrine solution on bisulfite concentration is evident from Fig. 3. The results shown are expressed in terms of observed reciprocal half-life since the kinetic order was relatively complex. If it is assumed that no true racemization occurs in presence of any finite concentration of bisulfite, then it would appear that the over-all dependency can be rationalized on the basis of a parallel zero-order and a first-order reaction with respect to the thio compound.

This picture is strongly supported by results shown in Figs. 4 and 5. For these plots the absolute initial rate of loss of bisulfite, followed iodometrically, has been determined as a function of bisulfite concentration in presence of 0.055 *M* *l*-epinephrine at *p*H 4.0 and 4.7. It is clearly evident that at the lower *p*H the rate of loss is essentially independent of concentration, *i.e.*, zero order. The concentration independent rate, 3.5×10^{-4} mole liter^{-1} hour^{-1}, shown can be corrected to pseudo first-order rate constant for loss of epinephrine by dividing this value by epinephrine concentration, $k = 3.5 \times 10^{-4}/0.055 = 6.3 \times 10^{-3}$ hr.$^{-1}$. The rate of racemization of *l*-epinephrine observed experimentally in absence of bisulfite under otherwise similar conditions was $k = 5.9 \times 10^{-3}$ hr.$^{-1}$.

These results indicate that bisulfite or sulfite tends to react rapidly with the activated form of

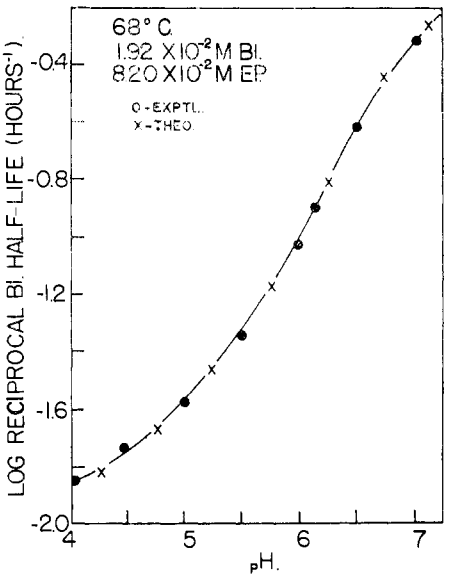


Fig. 6.—*p*H dependency of the pseudo first-order rate constant (proportional to reciprocal half-life) for bisulfite at 68° in various buffers. Solutions were 1.92×10^{-2} *M* with respect to sodium bisulfite (Bi) and 8.20×10^{-2} *M* with respect to epinephrine (Ep). Theoretical points calculated with expression V and $K_a = 10^{-7}$.

epinephrine through which the drug molecule must pass for it to racemize. From the data as little as 0.01 molar bisulfite is sufficient to compete successfully in preventing the formation of *d*-epinephrine.

Further insight into the mechanism of the over-all reaction is provided by data related to the *p*H dependency of the system. In Fig. 6 the observed half-life of bisulfite in the presence of a large excess of epinephrine has been determined as a function of *p*H. The curve has an approximate slope of one between *p*H 5.5 and 6.5, but lower values at the two extremes.

All the observed facts can be explained satisfactorily by the proposed mechanism

$$l\text{-Ep} \xrightarrow{k_1} \text{Ep}^* \xrightarrow{k} d\,l\text{-Ep}$$

$$\text{Ep}^* + \text{SO}_3^- \xrightarrow{k_4} \text{EpBi (racemic product)}$$

$$\text{Ep}^* \xrightarrow{k_3} \text{SO}_3^-$$

$$-\mathrm{d(Ep)}/dt = k_4(\text{Ep})(\text{SO}_3^-) + k_3(\text{Ep}^*)(\text{SO}_3^-) \quad \text{(I)}$$

$$\mathrm{d(Ep^*)}/dt = k_1(\text{Ep}) - k_2(\text{Ep}^*) - k_3(\text{Ep}^*)(\text{SO}_3^-) = 0 \quad \text{(II)}$$

$$(\text{Ep}^*) = \frac{k_1(\text{Ep})}{k_2 + k_3(\text{SO}_3^-)} \quad \text{(III)}$$

$$\frac{-\mathrm{d(Ep)}}{dt} = \frac{k_3(\text{SO}_3^-)\,k_1(\text{Ep})}{k_2 + k_3(\text{SO}_3^-)} + k_4(\text{Ep})(\text{SO}_3^-) \quad \text{(IV)}$$

In the above scheme it is suggested that the reaction with bisulfite or sulfite can occur by either of two routes. The simpler of these is the second-order reaction represented by k_4 involving direct attack by sulfite ion on the singly charged epinephrine molecule. Since it is assumed that sulfite ion is the reactant, this particular route shows a definite hydrogen ion dependency at *p*H values below 7. This accounts for the slope of approximately one

between *p*H 5.5 and 6.5. Above this value the *p*H dependency becomes smaller since the sulfite form becomes a sizable proportion of the total sulfite plus bisulfite in solution. Apparently rapid racemization subsequent to the rate-determining SN2 step leads to the racemic product since characteristic inversion of configuration at the asymmetric center is not observed.

The observed *p*H dependency between 5.5 and 6.5 can be equivalently explained on the basis that the catecholate form of the drug reacts with bisulfite ion. This possibility certainly cannot be ruled out on the basis of the present study.

The second route is thought to be mediated through formation of a carbonium ion which is normally responsible for racemization of the drug in absence of bisulfite. This reaction, governed by rate constant k_1, does not appear to be hydrogen ion dependent, at least in the *p*H range employed, since the racemization rate studied in absence of bisulfite indicated a half-life of 118 hours at *p*H 4.0 and 130 hours at *p*H 4.9 and 84.5°. The carbonium ion formed can react with water to form the racemic compound or in presence of bisulfite or sulfite react to form the sulfonate.

The suggested nucleophilic attack by sulfite (or bisulfite) ion on the carbonium ion (k_3) must be very fast compared to the rate of reformation of epinephrine. This assumption seems entirely logical in view of the species involved and explains the observed zero-order dependency on bisulfite provided k_1 governs the rate-determining step.

Below *p*H 6, and in the presence of bisulfite, the derived rate equation IV may be simplified to

$$-\mathrm{d(Ep)}/\mathrm{d}t = k_4(\mathrm{Ep})(\mathrm{SO_3^=}) + k_1(\mathrm{Ep}) \qquad (V)$$

Epinephrine, pK_a 8.55,[4] exists predominantly as positively charged species over the *p*H range studied and the second ionization constant of sulfurous acid, $K_{a_2} = 6.24 \times 10^{-8}$ at 25° in dilute solution, fixes the concentration of sulfite for any added stoichiometric quantity of sulfur compound: $(\mathrm{SO_3^=}) = S_{\mathrm{total}} K_a/(\mathrm{H^+})$ where $(\mathrm{H^+}) > K_a$.

In Fig. 6 theoretical points based on equation V and a K_a value of 1×10^{-7} are shown at widely spaced *p*H values. It is evident that the form of the equation is in good agreement with the observed experimental facts.[4]

Acknowledgment.—This study was supported in part by a grant from Parke, Davis and Co. of Detroit, Mich., and a grant from the Research Committee of the Graduate School from funds supplied by Wisconsin Alumni Research Foundation.

(4) M. M. Tuckerman, J. R. Mayer and F. C. Nachod, THIS JOURNAL, **81**, 92 (1959).

MADISON 6, WIS.

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH]

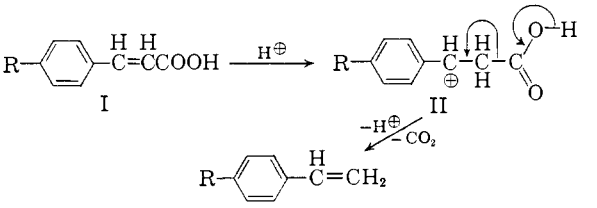
A Study of *p*H Dependence in the Decarboxylation of *p*-Hydroxycinnamic Acid[1]

BY LOUIS A. COHEN AND WILLIAM M. JONES

RECEIVED JULY 24, 1959

The rate of decarboxylation of *p*-hydroxycinnamic acid has been studied in aqueous media in the *p*H range 1–12. Of the several *p*-substituted cinnamic acids examined, the *p*-hydroxy compound is unusual in its ability to decarboxylate beyond *p*H 3. The rate of decarboxylation has been correlated with hydrogen ion concentration and with the concentrations of the various *p*H-dependent forms present in solution. Some evidence is presented to show that the principal decarboxylating species is the vinylog of the β-keto acid resulting from dienone–phenol tautomerism.

Although the thermal decarboxylation of substituted cinnamic acids is a common synthetic procedure,[2] decarboxylation in aqueous media has received only scant attention. The breakdown of *o*- and *p*-hydroxycinnamic acids by hot mineral acid was reported in 1889.[3] However, it was not until 1949 that a kinetic study of the influence of substituents on the rate of acid-catalyzed decarboxylation of cinnamic acids appeared.[4] Evidence then was presented that decarboxylation in strong mineral acid proceeds *via* an SE2 mechanism[5] since the rate increases continuously with increasing acid strength. A β-carbonium ion II was considered a likely intermediate, the decarboxylation being facilitated by β-substituents which tend either to increase the electron density at Cα or to stabilize the β-carbonium ion by resonance.



From a consideration of σ-constants, it might be expected that hydroxy- or methoxycinnamic acids would decarboxylate with particular ease and, indeed, the relatively rapid breakdown of a *p*-methoxycinnamic acid in hot mineral acid has been reported.[6] In the course of another investigation in this Laboratory, *p*-hydroxycinnamic acid was found to be unstable, decarboxylation being observed over a wide *p*H range.

Buffered solutions of *p*-hydroxycinnamic acid (0.02 *M*) were decarboxylated at 100° at various

(1) Second paper of a series on phenol–dienone tautomerism; for paper I, *cf.* L. A. Cohen, *J. Org. Chem.*, **22**, 1333 (1957).

(2) (a) T. W. Abbott and J. R. Johnson, "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1948, p. 440; (b) C. Walling and K. B. Wolfstirn, THIS JOURNAL, **69**, 852 (1947).

(3) W. v. Miller and F. Kinkelin, *Ber.*, **22**, 1715 (1889).

(4) W. S. Johnson and W. E. Heinz, THIS JOURNAL, **71**, 2913 (1949).

(5) H. Schenkel and M. Schenkel-Rudin, *Helv. Chim. Acta*, **31**, 514 (1948).

(6) W. S. Johnson and M. W. Miller, THIS JOURNAL, **72**, 511 (1950).